

APPENDIX B

Pending Claims Upon Entry of Instant Amendment

1. A method of regulating endothelial cell growth, comprising the step of contacting endothelial cells with a composition comprising a purified polypeptide in an amount effective to regulate endothelial cell growth, wherein said polypeptide:

(a) binds the extracellular domain of Flt4 receptor tyrosine kinase and stimulates Flt4 autophosphorylation;

(b) has an apparent molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions; and

(c) has an amino acid sequence comprising a portion of SEQ ID NO: 8 effective to permit binding to the Flt4 extracellular domain.

2. A method according to claim 1, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 5.

3. A method according to claim 1, wherein said polypeptide is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

4. A method according to claim 1 wherein the endothelial cells are lymphatic endothelial cells.

5. (Amended) A method of modulating the activity of Flt4 receptor tyrosine kinase (Flt4), comprising the steps of:

identifying a patient in need of modulation of Flt4 activity; and

administering to the patient a composition comprising a purified polypeptide in an amount effective to modulate the activity of Flt4, wherein the polypeptide binds the extracellular domain (EC) of Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4, said polypeptide comprising an

amino acid sequence comprising a portion of SEQ ID NO: 8 effective to permit such binding.

7. A method according to claim 5, wherein the composition further comprises a pharmaceutically-acceptable diluent, adjuvant, or carrier.

8. A method according to claim 5, wherein the identifying step comprises identifying a patient suffering from a disorder of the lymphatic system, and wherein the polypeptide is administered in an amount effective to modulate Flt4 activity in endothelial cells of lymphatic vessels of the patient.

9. A method according to claim 5, wherein the polypeptide binds Flt4 and promotes proliferation of lymphatic endothelial cells that express Flt4.

11. A method according to claim 5, wherein the polypeptide comprises a contiguous portion of SEQ ID NO: 8 that is sufficient to bind human Flt4EC,

wherein said contiguous portion includes eight cysteine residues that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factor A (PDGF-A), and human platelet derived growth factor B (PDGF-B), and

wherein said polypeptide lacks any portion of SEQ ID NO: 8 that has one or more cysteine motifs of a Balbiani ring 3 protein (BR3P).

12. A method according to claim 5, wherein the polypeptide comprises a portion of the amino acid sequence in SEQ ID NO: 8 effective to permit said binding to the Flt4 extracellular domain, said polypeptide lacking at least carboxy-terminal residues of SEQ ID NO: 8 beyond residue 227.

13. A method according to claim 5, wherein the polypeptide is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC Accession Number CRL 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

14. A method according to claim 5, wherein the polypeptide has an amino acid sequence consisting of a portion of the amino acid sequence set forth in SEQ ID NO: 8, said portion including from residue 161 of SEQ ID NO: 8 to residue 211 of

SEQ ID NO: 8, said portion lacking at least carboxy-terminal residues of SEQ ID NO: 8 beyond residue 227.

15. A method according to claim 14, wherein the portion of the amino acid sequence set forth in SEQ ID NO: 8 includes from residue 131 of SEQ ID NO: 8 to residue 211 of SEQ ID NO: 8.

16. A method according to claim 14, wherein the portion of the amino acid sequence set forth in SEQ ID NO: 8 includes from residue 113 of SEQ ID NO: 8 to residue 213 of SEQ ID NO: 8.

17. A method according to claim 14, wherein the portion of the amino acid sequence set forth in SEQ ID NO: 8 includes amino acids 103 to 217 of SEQ ID NO: 8.

18. A method according to claim 14, wherein the portion of the amino acid sequence set forth in SEQ ID NO: 8 includes amino acids 32 to 227 of SEQ ID NO: 8.

19. (Amended) A method of modulating the activity of Flt4 receptor tyrosine kinase (Flt4) in Flt4-expressing cells, comprising the steps of:

(a) preparing a polynucleotide comprising a nucleotide sequence that encodes a polypeptide that binds to the extracellular domain of human Flt4,

wherein said polynucleotide includes a strand that hybridizes to a DNA comprising the non-coding strand complementary to SEQ ID NO: 32, under the following hybridization conditions:

(i) hybridization at 42°C for 20 hours in a solution containing 50% formamide, 5x SSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA; and

(ii) washing the filter twice for thirty minutes at room temperature and twice for thirty minutes at 65°C with a wash solution containing 1x SSC, and 0.1% SDS;

(b) transforming or transfecting a cell with the polynucleotide such that the cell expresses and secretes a polypeptide encoded by said polynucleotide, wherein said secreted polypeptide binds the extracellular domain of human Flt4, stimulates Flt4 autophosphorylation, and has a molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions; and

(c) contacting Flt4-expressing cells with the secreted 23 kD polypeptide.

20. A method according to claim 19, wherein the polypeptide has an amino acid sequence comprising a continuous portion of the amino acid sequence shown in SEQ ID NO: 8 effective to permit said binding.

21. A method according to claim 19, wherein the polynucleotide comprises a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO: 8, wherein the polynucleotide is transcribed and translated in the cell to produce a prepro-VEGF-C polypeptide having the amino acid sequence shown in SEQ ID No: 8, and wherein the prepro-VEGF-C polypeptide is proteolytically processed to form the 23 kD polypeptide.

22. A method according to claim 19, wherein the polynucleotide comprises the polypeptide-encoding insert of plasmid pFLT4-L, deposited as ATCC Accession No. 97231.

23. A method according to claim 19, wherein the polynucleotide further includes an expression control sequence operably linked to the sequence that encodes the polypeptide.

24. A method according to claim 19, wherein the transforming step comprises contacting the cell with vector that contains the polynucleotide.

25. A method according to claim 19, wherein the Flt4-expressing cells are human endothelial cells.

26. A method according to claim 25, wherein the human endothelial cells are lymphatic endothelial cells.

27. A method according to claim 25, wherein steps (b) and (c) are performed in vivo.

28. (Amended) A method of stimulating the proliferation of mammalian endothelial cells comprising the step of contacting mammalian endothelial cells with a composition comprising a polypeptide in an amount effective to modulate the proliferation of mammalian endothelial cells, said polypeptide comprising a VEGF-C Δ C156 polypeptide that binds to human Flt4 receptor tyrosine kinase (Flt4) and fails to bind to human KDR receptor tyrosine (VEGFR-2), said polypeptide having an

amino acid sequence comprising a portion of SEQ ID NO: 8 effective to permit binding to Flt4, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced by another amino acid.

29. A method according to claim 28, wherein the portion of SEQ ID NO: 8 is selected from the group consisting of:

(a) a continuous portion having as its amino terminal residue an amino acid between residues 102 and 114 of SEQ ID NO: 8 and having as its carboxy terminal residue an amino acid between residues 212 and 228 of SEQ ID NO: 8, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced by another amino acid;

(b) continuous portions that comprise an amino-terminal truncation of (a);
and

(c) continuous portions that comprise a carboxyl-terminal truncation of (a) or (b).

30. A method according to claim 28, wherein said endothelial cells are lymphatic endothelial cells.

31. (Amended) An in vivo method according to claim 28, wherein the contacting step comprises administering to a mammalian subject in need of stimulation of the growth of lymphatic endothelial cells a composition comprising said polypeptide, in an amount effective to stimulate the growth of lymphatic endothelial cells in vivo.

32. A method according to claim 31, wherein said polypeptide has reduced effect on the permeability of mammalian blood vessels compared to a wildtype VEGF-C polypeptide with an amino acid sequence set forth in SEQ ID NO: 8 from residue 103 to residue 227.

33. (Amended). A method of stimulating the proliferation of mammalian endothelial cells comprising the step of contacting mammalian endothelial cells with a composition comprising a polypeptide in an amount effective to stimulate the proliferation of mammalian endothelial cells, said polypeptide comprising a fragment of a vertebrate prepro-VEGF-amino acid sequence that binds to human Flt4 receptor

tyrosine kinase, with the proviso that, in said polypeptide, a conserved cysteine of the vertebrate prepro-VEGF-C has been deleted or replaced by another amino acid,

wherein the vertebrate prepro-VEGF-C amino acid sequence comprises an amino acid sequence that is encoded by a DNA of vertebrate origin which hybridizes to a non-coding strand complementary to nucleotides 352 to 1611 of SEQ ID NO: 7 under the following hybridization conditions: hybridization at 42°C in a hybridization solution comprising 50% formamide, 5 X SSC, 20 mM Na₂PO₄, pH 6.8; and washing in 0.2 X SSC at 55°C,

wherein nucleotides 352 to 1611 of SEQ ID NO: 7 encode a human prepro-VEGF-C having the amino acid sequence set forth in SEQ ID NO: 8 that is characterized by eight cysteine residues at positions 131, 156, 162, 165, 166, 173, 209, and 211 of SEQ ID NO: 8 that are conserved in human vascular endothelial growth factor (VEGF), human platelet derived growth factors A and B (PDGF-A, PDGF-B), human placenta growth factor (PlGF-1), and human vascular endothelial growth factor B (VEGF-B), and

wherein the conserved cysteine that has been deleted or replaced corresponds to position 156 of SEQ ID NO: 8.

34. A method according to claim 33, wherein the vertebrate is a human.

35. A method according to claim 33, wherein the vertebrate is a mouse.

36. A method according to claim 33, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO: 8, wherein the cysteine residue at position 156 of SEQ ID NO: 8 has been deleted or replaced by another amino acid;

(b) the amino acid sequence of SEQ ID NO: 11, wherein the cysteine residue at position 152 of SEQ ID NO: 11 has been deleted or replaced by another amino acid;

(c) the amino acid sequence of SEQ ID NO: 13, wherein the cysteine residue at position 155 of SEQ ID NO: 13 has been deleted or replaced by another amino acid;

(d) amino-terminal truncations of (a), (b), or (c); and

(e) carboxyl-terminal truncations of (a), (b), (c), or (d).

37. (Amended) An in vivo method according to claim 33, wherein the contacting step comprises administering to a mammalian subject in need of stimulation of the growth of lymphatic endothelial cells a composition comprising said polypeptide, in an amount effective to modulate the growth of lymphatic endothelial cells in vivo.

40. A method according to claim 19, further comprising a step of purifying the secreted polypeptide prior to the contacting step.